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# PIP3, PIP2, and Cell Movement—Similar Messages, Different Meanings?

Commentary

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**The inositol lipids PI(4,5)$P_2$ and PI(3,4,5)$P_3$ are important regulators of actin polymerization, but their different temporal and spatial dynamics suggest that they perform separate roles. PI(3,4,5)$P_3$ seems to act as an instructive second messenger, inducing local actin polymerization. PI(4,5)$P_2$ appears to be present at too high a concentration and homogenous a distribution to fulfil a similar role. Instead, we suggest that PI(4,5)$P_2$ acts permissively, restricting new actin polymerization to the region of the plasma membrane.**

## Introduction

The movement, polarity, and shape of eukaryotic cells together constitute one of the most exciting areas of modern cell biology. Recent months have seen a spectacular increase in our understanding of the way these processes are controlled, both during normal cellular housekeeping and in response to extracellular signals. In particular, the inositol lipids PI(4,5)$P_2$ (phosphatidylinositol 4,5-bisphosphate) and PI(3,4,5)$P_3$ (phosphatidylinositol 3,4,5-trisphosphate) have emerged as important regulators of the cytoskeleton and cell motility in response to multiple signals and in a wide variety of cells. Clearly, both are major signals to the actin cytoskeleton, but what sorts of messages do these lipids convey? Recent advances in analyzing the spatial and temporal dynamics of lipid production have yielded important clues to how cells actually use PI(4,5)$P_2$ and PI(3,4,5)$P_3$ to orchestrate actin polymerization and cell motility. In this article, we argue that PI(4,5)$P_2$ and PI(3,4,5)$P_3$ are complementary signals that carry different messages to the cytoskeleton. While PI(3,4,5)$P_3$ is instructive—changes in PI(3,4,5)$P_3$ levels can specify both spatial and temporal dynamics of actin polymerization—we suggest that PI(4,5)$P_2$ has a different, permissive role. While PI(4,5)$P_2$ regulates the activities of several different proteins that control actin dynamics, it seems its levels rarely change enough to cause a major shift in cytoskeletal behavior. However, nearly all actin polymerization occurs in the cell cortex, immediately adjacent to the cell surface, and PI(4,5)$P_2$ is largely restricted to the plasma membrane. We therefore suggest that PI(4,5)$P_2$ acts to restrict actin polymerization to the cortex. In contrast, PI(3,4,5)$P_3$ appears to be able to control when and in which part of the cortex actin polymerization is initiated.

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## PIP3—An Instructive Signal for Actin Polymerization

PI(3,4,5)$P_3$ fulfils all the roles expected of a second messenger that instructively couples cell signaling to actin polymerization. PI(3,4,5)$P_3$ levels are tightly regulated and appropriate for the mechanisms that are thought to mediate its functions. In most cells, the kinetics of PI(3,4,5)$P_3$ production closely parallel those of stimulus-induced actin polymerization (Figure 1A). In neutrophils, levels of PI(3,4,5)$P_3$ are very low in unstimulated cells, but they increase dramatically within 10 s of stimulation with chemoattractant (Stephens et al., 1991), closely coinciding with the kinetics of actin polymerization (Howard and Oresajo, 1985). Concentrations change from approximately 50 nM in resting cells to 2 μM 10 s after stimulation (Stephens et al., 1991), corresponding to local concentrations at the membrane of 5 μM and 200 μM, respectively.

Furthermore, in a range of cell types, this burst of PI(3,4,5)$P_3$ appears to couple signaling to actin polymerization. Pharmacological inhibitors of PI(3,4,5)$P_3$ generation, dominant-negative proteins, and mutant receptors unable to activate PI 3-kinases have been found to interfere with stimulus-induced actin polymerization in cells from neutrophils (Niggli and Keller, 1997) to fibroblasts (Wennstrom et al., 1994) to *Dictyostelium* (Funamoto et al., 2001). While PI(3,4,5)$P_3$ is clearly not always required for actin polymerization (see, for example, Kovacsovics et al., 1995), when this lipid is produced, it is sufficient to cause nucleation of new actin filaments in most cells. Consistent with this view, artificial increases in PI(3,4,5)$P_3$ levels through activated versions of PI 3-kinases or direct lipid delivery to cells (Derman et al., 1997; Niggli, 2000) stimulate actin polymerization and in some cases cell polarity and motility.

If PI(3,4,5)$P_3$ is an instructive cue for actin polymerization, we would also expect its spatial distribution to parallel that of actin polymerization. Analysis of the dynamics of PI(3,4,5)$P_3$ in a number of motile cells confirms this prediction (Figure 1B). Several groups have used PI(3,4,5)$P_3$-specific pleckstrin homology (PH) domains fused to GFP to probe the localized changes in PI(3,4,5)$P_3$ induced by extracellular signals. During chemotaxis of neutrophils (Servant et al., 2000), *Dicytostelium* (Parent et al., 1998; Meili et al., 1999), and fibroblasts (Haugh et al., 2000), the spatial distribution of PI(3,4,5)$P_3$ closely matches that of actin polymerization. Unstimulated cells lack detectable recruitment of PI(3,4,5)$P_3$—binding PH domains to the plasma membrane. Upon stimulation with a gradient of chemoattractant, the cells polymerize actin in a polarized fashion aligned with the external gradient, and PI(3,4,5)$P_3$ exhibits the same polarity. Therefore, PI(3,4,5)$P_3$ is not only sufficient to induce actin polymerization, but it is also generated at the right time and the right place to instruct when and where actin polymerization will occur.

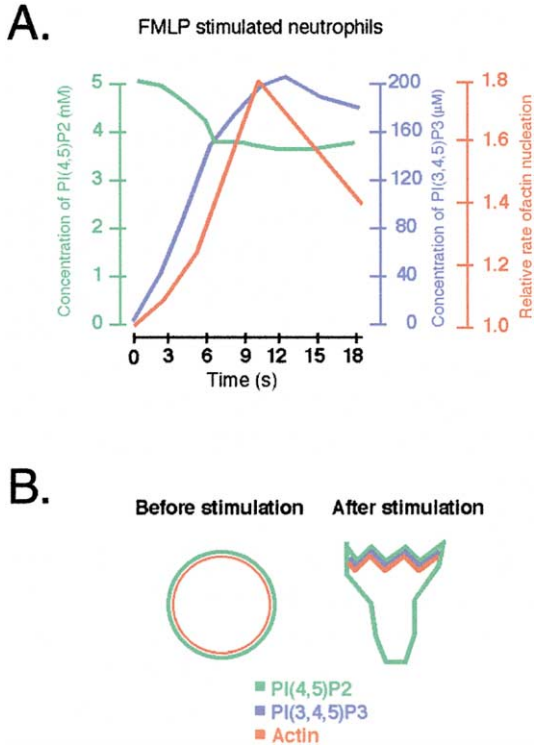


Figure 1. Temporal and Spatial Changes in $PI(4,5)P_2$, $PI(3,4,5)P_3$, and Actin Polymerization

(A) Time course of changes in $PI(4,5)P_2$, $PI(3,4,5)P_3$, and actin polymerization in fMLP-stimulated neutrophils. $PI(4,5)P_2$, $PI(3,4,5)P_3$ data refer to the approximate plasma membrane concentrations. $PI(3,4,5)P_3$ and new actin nucleation behave similarly, but $PI(4,5)P_2$ levels decrease (adapted from Stephens et al., 1991, 1993; Carson et al., 1986). This response is representative of many cell types.

(B) Spatial distribution of $PI(4,5)P_2$, $PI(3,4,5)P_3$, and actin polymerization in cells before stimulation and during chemotaxis, as revealed by GFP-tagged PH domains and phalloidin. Prior to stimulation, $PI(4,5)P_2$ and F-actin are uniformly distributed throughout the cortex with undetectable $PI(3,4,5)P_3$. After stimulation, $PI(4,5)P_2$ remains uniformly distributed, but actin and $PI(3,4,5)P_3$ accumulate at the leading edge of the cell (reviewed in Rickert et al., 2000).

How is $PI(3,4,5)P_3$ generation coupled to actin polymerization? This is not yet proven, but the Rho family GTPases Cdc42 and Rac are good candidates. The guanine nucleotide exchange factors (GEFs) involved in activation of Rho family proteins are characterized by a tandem Dbl homology domain, where the GEF activity lies, and an inositol lipid binding PH domain. Vav, a GEF for the Rho family, exhibits decreased exchange activity in the presence of $PI(4,5)P_2$ and increased activity in the presence of $PI(3,4,5)P_3$ (Han et al., 1998). Other GEFs for Rac, such as Sos and Pix, show a similar dependence on $PI(3,4,5)P_3$ for their activation (Nimnual et al., 1998; Yoshii et al., 1999). Importantly, the levels of $PI(3,4,5)P_3$ in resting and stimulated cells are within the expected range needed for proper recruitment and activation of these exchange factors. Consistent with these results, Cdc42 and Rac activation in neutrophils and Rac activation in fibroblasts are inhibited if $PI(3,4,5)P_3$ increases are blocked pharmacologically (Hawkins et al., 1995; Benard et al., 1999), and PI 3-kinase-dependent actin rearrangements in fibroblasts are dependent on Rac activation. Cdc42 stimulates actin polymerization though the Wiskott-Aldrich Syndrome protein (WASP) and its relatives, which cause polymerization by activating the Arp2/3 complex. Taken together, these data suggest that $PI(3,4,5)P_3$ stimulates actin polymerization by the recruitment and activation of Rho GTPases such as Rac and Cdc42, which in turn stimulate the nucleation of actin polymerization, thereby planting the seeds from which new actin filaments will grow.

## PIP2—A Message, but What Does It Mean?

The role of $PI(4,5)P_2$ in the control of cytoskeletal processes is less clear-cut. Superficially, $PI(4,5)P_2$ has many similarities to $PI(3,4,5)P_3$ in its effects on actin polymerization. Sequestration of $PI(4,5)P_2$ can inhibit actin polymerization in platelets (Hartwig et al., 1995) and neutrophils (Glogauer et al., 2000). Furthermore, artificially increasing $PI(4,5)P_2$ levels, through direct delivery or overexpression of the kinases that are thought to generate it, has been found to induce actin polymerization in several cell types. However, observations of this type are not enough to implicate this lipid as a direct instructive signal to the actin cytoskeleton if it is not generated in the right place and at the right time. Levels of $PI(4,5)P_2$, unlike $PI(3,4,5)P_3$, are relatively high in all cells, stimulated or unstimulated. Furthermore, the changes induced by signaling are variable between cell types, and there is often a poor correlation between $PI(4,5)P_2$ levels and changes in actin dynamics. Taken together, these points make it hard to envision $PI(4,5)P_2$ as an instructive second messenger in the same way as $PI(3,4,5)P_3$.

$PI(4,5)P_2$ is an essential component of eukaryotic cell membranes. It is required for the activity of several other signaling systems, in particular the many isoforms of phospholipase C and $PI_3$ kinases themselves. It is therefore unsurprising that most cells contain substantial concentrations of $PI(4,5)P_2$, irrespective of extracellular signals. For example, unstimulated neutrophils, which are round and move very little, contain approximately 50 μM total $PI(4,5)P_2$, which corresponds to a concentration of 5 mM at the inner leaflet of the plasma membrane (Stephens et al., 1991). It is hard to envisage such a high concentration constituting a specific or localized signal.

A further difficulty with $PI(4,5)P_2$ as an instructive cytoskeletal signal is that changes in its levels are very variable between cell types and signals. In neutrophils, for example, stimulation with fMLP causes a small drop in $PI(4,5)P_2$ concentrations (Stephens et al., 1991), despite a roughly 2-fold increase in the amount of polymerized actin (Howard and Oresajo, 1985). Several groups have used $PI(4,5)P_2$-specific PH domains fused to GFP to probe the localized changes in $PI(4,5)P_2$ induced by signals. During chemotaxis of neutrophils and *Dictyostelium*, actin polymerization is highly polarized. In contrast, $PI(4,5)P_2$-specific PH-GFP fusions are uniformly distributed around the plasma membrane of these cells, both before stimulation and during chemotaxis. Overall, it seems that agonists that stimulate motility may raise or lower $PI(4,5)P_2$ levels or even leave them largely unchanged. This is hard to reconcile with mechanisms in which changes in $PI(4,5)P_2$ levels directly cause actin polymerization.

One argument has been widely used to reconcile the

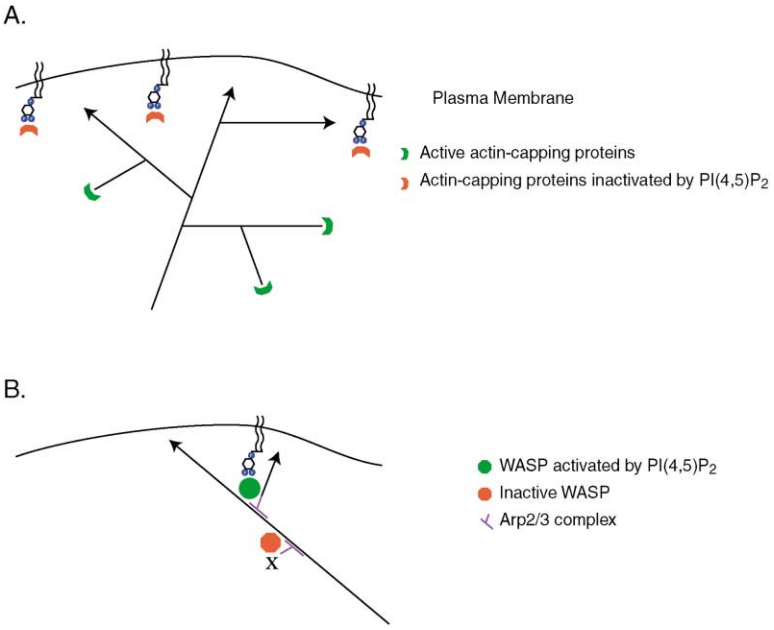


Figure 2. Potential Molecular Targets of $PI(4,5)P_2$ at the Plasma Membrane

(A) Local inactivation of capping proteins. Actin filaments which extend toward the cell body are capped by various F-actin capping proteins. Immediately below the membrane these proteins are inactivated by binding to $PI(4,5)P_2$.

(B) Local activation of WASP family proteins. Binding of WASP family proteins, in concert with the Arp2/3 complex, to existing filaments is required for new filament branching. WASP can only be activated by $PI(4,5)P_2$ when it is bound to the plasma membrane. This ensures that newly nucleated filaments are always oriented toward the membrane and away from the cell body.

large and relatively invariant pool of $PI(4,5)P_2$ with the dynamic and controlled behavior of actin. If most of the $PI(4,5)P_2$ in the plasma membrane is tightly bound to one or more sequestering proteins, which prevent it from interacting with signaling proteins, then only newly synthesized $PI(4,5)P_2$ would induce downstream effects. We find this argument unconvincing on kinetic grounds. A pool of sequestering proteins would need both high affinity and an abundance greater than membrane $PI(4,5)P_2$ levels to function. However, a high enough concentration of free-sequestering proteins—sufficient to obscure the plasma membrane completely—would tend to bind newly synthesized $PI(4,5)P_2$ faster than signaling proteins and, therefore, remove the putative signals just as effectively as the background of membrane $PI(4,5)P_2$. For a sequestering protein to block the effects of bulk $PI(4,5)P_2$, it would need highly specialized properties (for example, a very slow on rate and an extremely slow off rate), and as far as we know no such protein has been described.

These kinetic arguments are supported by studies with GFP-tagged PH domains that bind $PI(4,5)P_2$. These are usually localized at the cell surface before and after stimulation. Since these PH domains bind within the range of concentrations thought to constitute a signal, this also indicates that there is usually a significant amount of unbound $PI(4,5)P_2$ at the plasma membrane.

One possible conflict with our argument comes from platelets, in which $PI(4,5)P_2$ is thought to activate actin polymerization directly. Platelets have a high baseline level of $PI(4,5)P_2$—200 $\mu$M in the resting state, which initially decreases after TRAP stimulation but then, in contrast to most cells, increases beyond its baseline to approximately 250 $\mu$M during the time that actin polymerization is taking place (Hartwig et al., 1995). This increase is thought to be responsible for uncapping actin filaments, leading to new polymerization. Inhibition of $PI(3,4,5)P_3$ synthesis does not affect the stimulation of actin polymerization by TRAP (Kovacsovics et al., 1995). However, this appears to be more the exception than the rule. For most other cells that exhibit stimulus-induced actin polymerization, $PI(4,5)P_2$ levels decrease upon stimulation, $PI(3,4,5)P_3$ increases precede new actin polymerization, and $PI(3,4,5)P_3$ increases are necessary for new actin polymerization. This is not the case for platelets.

### PIP3 as a Switch and PIP2 as a Signpost

All the evidence currently available suggests that $PI(3,4,5)P_3$ is an instructive signal—production of this lipid determines where and when actin polymerization is to take place. As described above, we do not believe that such a role is likely for $PI(4,5)P_2$. Since $PI(4,5)P_2$ is clearly involved in regulating actin dynamics, what alternative physiological roles might it fulfil?

Observation of GFP-PH domain fusions reveals one relevant aspect of cell behavior. Even though cells are full of membrane-bound structures, $PI(4,5)P_2$-specific markers overwhelmingly localize to the plasma membrane at the surface of the cell. Nucleus, Golgi, and other vesicular structures only rarely show enrichment of the marker. The same is to a large degree true of actin structures—nearly all are formed at the plasma membrane. This is even clearer when the sites of actin nucleation, at which new actin filaments are formed, are examined. Actin nuclei are essentially all located just beneath the plasma membrane. New actin monomers are added between the nuclei and the membrane, and the filaments thus formed are swept into the cell body by rearward flow and disassembled within the cell. Any disruption of this pattern has serious consequences for cell motility.

Another, related, key feature of the actin cytoskeleton is that filaments near the surface are aligned outward. Actin moves cells by intercalating new monomers between plasma membrane and the existing actin cortex and thus pushing the membrane forward. This requires that actin filaments be oriented with their barbed, growing ends toward the cell surface. It is currently believed

that lamellipodia are formed by nucleation of new branches at a 70° angle off existing actin filaments (reviewed in Mullins, 2000). Unless it is tightly regulated, this process will tend to scramble the direction in which newly formed actin filaments extend; a mere three branchings in the same direction will extend new actin filaments toward the center of the cell. Since this is almost never seen, some signal must bias growth of new actin filaments toward the membrane. $PI(4,5)P_2$ is an attractive candidate for this signal due to its plasma membrane distribution and its interaction with actin binding proteins. In this model, $PI(4,5)P_2$ does not act as an instructive signal, or cells would exist in a constant, futile state of activation. Rather, it provides a spatial cue—a marker of the plasma membrane, but it is a marker that is always on, relying on its distribution rather than dynamics to regulate actin polymerization.

When other factors—including $PI(3,4,5)P_3$, small GTPases, heterotrimeric G proteins, and protein kinases—induce actin nucleation, $PI(4,5)P_2$ regulation would ensure that actin polymerization only occurs at the plasma membrane, and the resulting filaments are oriented outward. In other words, $PI(3,4,5)P_3$ is a switch that turns on the polymerization of actin filaments, and $PI(4,5)P_2$ is a marker that ensures they grow at the right site and in the right direction. Both signals are required for proper actin regulation, but it is the timing and location of the switch that ultimately determines where and when actin polymerization is to take place.

### Connections between PIP2 and the Cytoskeleton

How could a $PI(4,5)P_2$ signpost control the actin cytoskeleton? We summarize two possible mechanisms in Figure 2. Neither is essential to the underlying argument—that $PI(3,4,5)P_3$ is a switch and $PI(4,5)P_2$ a marker—and others will undoubtedly turn out to be important. These models are therefore presented to show the types of mechanism that cells could use.

One well-characterized pathway is the interaction of $PI(4,5)P_2$ with a variety of capping proteins. These proteins bind to the fast growing end of filaments and prevent further growth of the actin filament. $PI(4,5)P_2$ can displace these capping proteins, thereby enabling elongation of existing actin filaments. Because the ultimate length of newly nucleated actin filaments is dependent on their rate of capping, $PI(4,5)P_2$ has the potential to bias the orientation of actin filaments through a simple selection—it could act to inhibit capping of newly nucleated actin filaments but only if the direction of their growth is oriented toward the plasma membrane [where the growing end of the actin filament is capable of interacting with $PI(4,5)P_2$].

$PI(4,5)P_2$ also has a clear role in the initiation of new actin filaments. Recent studies by a number of labs have shown that $PI(4,5)P_2$ modulates nucleation of actin polymerization through its interaction with the WASP family of proteins. In vitro, $PI(4,5)P_2$ synergizes with Cdc42 in activation of WASP family proteins, leading to nucleation of actin polymerization through the Arp2/3 complex (reviewed in Fawcett and Pawson, 2000). If WASP activation and actin nucleation require coincident activation of Cdc42 and plasma membrane distributed $PI(4,5)P_2$, this would help limit actin nucleation to the plasma membrane with tighter spatial regulation than if WASP were regulated by Cdc42 activation alone. Again, it is important to emphasize that we do not propose that $PI(4,5)P_2$ increases instructively regulate actin nucleation—its spatial and temporal dynamics appear inappropriate to do so—rather, we suggest that the primary role of $PI(4,5)P_2$ for these actin rearrangements is to denote the location of the plasma membrane.

## Conclusion

The weight of evidence suggests that $PI(3,4,5)P_3$ is a second messenger in the stimulation of actin polymerization and cell polarity. While cells undoubtedly use many other mechanisms to control actin dynamics, a rise in $PI(3,4,5)P_3$ levels is alone sufficient to induce actin assembly, and the kinetics and spatial dynamics of $PI(3,4,5)P_3$ generation correlate well with that of stimulus-induced actin polymerization. It therefore clearly seems to be an instructive signal, and a full understanding of what controls the location and level of $PI(3,4,5)P_3$ promises to yield extremely informative advances in cell motility. This is not true for $PI(4,5)P_2$, which is present at such high and homogenous levels in most cells that it is unlikely to work instructively. Instead, we believe that $PI(4,5)P_2$ has an equally crucial, but different role. Since it is tightly localized within the cell, it could restrict actin polymerization to the area immediately beneath the plasma membrane, thereby ensuring the actin filaments (whether initiated in response to $PI(3,4,5)P_3$ or other signals) grow in the right direction to produce membrane extension and cell motility. The instructive information provided by $PI(3,4,5)P_3$ would cooperate with the plasma membrane marker provided by $PI(4,5)P_2$ to provide a robust and flexible system to control actin dynamics and thus regulate multiple aspects of cell motility and behavior.

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